SOME EFFECTS OF MALATHION AND EPN ON MICE

by

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TABLE OF CONTENTS

INTRODUCTION AND REVIEW OF LITERATURE

During the modern time much work has been done on chemical control of insects. In addition to insects, mammals in many instances are exposed to insecticides either by direct contact or through residues in food. Since man may be exposed innocently to insecticides the Food and Drug Administration requires various mammalian toxicity tests (Lehman, et al., 1955) to be made on a compound before it can be considered for labeling and sale.

During the last few years organic phosphorus insecticides have become popular and many mammalian studies are being done with these compounds. One of the insecticides of interest is malathion because of its wide use and low mammalian toxicity. Holland, et al. (1952) determined LD_{50}'s of different grades and found the purer grades to be less toxic. They also found that rats tolerated satisfactorily 1000 p.p.m. malathion continuously in their diet.

The other insecticide considered in this manuscript is EPN. EPN is more toxic to mammals than malathion. Hodge, et al. (1954) did some toxicity work on EPN and found that female rats were more susceptible than males to acute intoxication. They found also that there were no histological changes in rats' organs when chronically fed.

It is possible that a mixture of insecticides can have more than their added toxicity (potentiation). Frawley, et al. (1957) found potentiation between malathion and EPN in rats and dogs measured by blood cholinestrase depression. They determined an LD_{50} combination in rats of about 165 mg. malathion plus 6.6 mg. EPN per kg. Cook, et al. (1958) explained biochemically that malathion and EPN potentiation was caused by oxidized EPN inhibiting malathionase, a malathion detoxification enzyme. To confirm some phases of malathion-EPN potentiation, an LD_{50} of mice with this combination was determined in this study.

Lehman, et al. (1955) mentioned that in addition to chronic feeding tests on mammals, reproductive studies could be made. It is believed that the added load of stresses of growth, pregnancy, and lactation may increase the toxicity of a compound. This study included reproductive studies which were made on mice using malathion and in one case a malathion EPN combination to determine if fertility would be decreased and if litter survival and growth would be altered. Both an acute and a chronic study were conducted.

MATERIALS AND METHODS

Maintenance and Identification of Experimental Animals

The experimental animal used was the white mouse (Mus musculus L.) of the Swiss strain. The mice were purchased at six weeks of age from Huntingdon Farms in Philadelphia, Pennsylvania and were shipped by Air Express in screen-sided wood boxes supplied with feed, potatoes, and wood shavings.

Upon arrival the mice were transfered to cages in the mouse rearing room. The mouse rearing room, a small windowless room, was kept at a fairly constant temperature (25 to 27° C.) by an air conditioner and an electric heater both regulated by a thermostat. The cages were steel sided with a 1/2 inch screen front and bottom with an open top. The cages were the drawer type on a steel rack holding 60 cages. Under the cages were large trays filled with wood shavings which were replaced three times a week.

The mice were provided with an ample supply of Purina Laboratory Chow. They were supplied water by a water bottle fastened upside down to the front of the cage. The water bottle was a 140 ml. rubber stoppered bottle with a slightly bent 8 mm. glass tube through the rubber stopper.

The mice were identified by clipping off certain toes with a sissors at

least one day before dosing. The numbering system used was a slightly modified form (Fig. 1) of the system mentioned by Eaton and Cabell (1949).

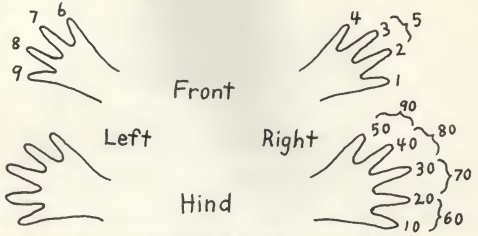

Fig. 1. Diagram of feet showing system of numbering used in these experiments. The left hind foot could be used if numbering into the hundreds was desired.

Before treatment the mice were transferred to large mouth gallon jars (sometimes up to seven mice per jar) with a 3/8 inch screen top (Fig. 2). About 1/2 inch of wood shavings and an ample supply of Purina Laboratory Chow were placed at the bottom of the jar. The water bottle rested on top of the jar with its glass tube (slightly bent near the end) going through the mesh of the screen, the tip of the glass tube being about three inches from the bottom of the jar. These jars containing mice were kept in the mouse rearing room after treatment until the observation period was over and then the mice were disposed of.

Insecticides and Solvent

The malathion, 0,0-dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate, was of technical grade of 95 per cent purity and was obtained from the American Cyanamid Company. The malathion was stored in a sealed bottle kept in a refrigerator.

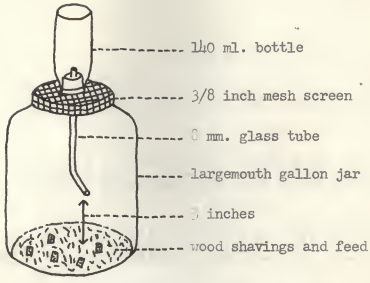

Fig. 2. Diagram of jar used to hold treated mice.

The EPN, O-ethyl-O-p-nitrophenyl benzenethiophosphate, was obtained from E. I. Du Pont de Nemours and Co. in technical grade which is a dark thick liquid. Since the pure crystalline form (light yellow) was needed the technical EPN was purified by crystallization. The crude EPN was dissolved in a warm 1:1 mixture of isopropyl and methyl alcohol. Decolorizing carbon was added and filtered off. The solution was cooled and then seeded with some pure EPN crystals (obtained from company previously) and then placed in a refrigerator overnight during which time the product crystallized out. The crystals were filtered off and recrystallized two more times by dissolving them in fresh solvent (1:1 isopropyl-methyl alcohol mixture), filtering the solution, cooling and seeding it, and then placing it in a refrigerator over night. The crystalline EPN, after being dried, was kept in a desiccator at room temperature. The melting point of this EPN was 36° C. which compares with the value of 36° C. given by Martin (1955).

The solvent used to dissolve and dilute these insecticides for dosing was Mazola oil (corn oil). It was purchased in pint quantities from a local

supermarket. The oil was kept at room temperature. It was checked occasionally for acidity with dilute NaOH and phenolphthalein since malathion is unstable in acid solution.

Preparation of Solutions

The insecticide solutions were made up in 10 ml. or 5 ml. glass stoppered volumetric flasks. When working with one insecticide alone the solution was made up so that the average volume of the dose per mouse 0.1 ml. When working with the combination of insecticides the two solutions were made up separately so that the average volume of solution per dose of each insecticide was 0.05 ml. All the glassware and equipment used in making solutions were rinsed several times with technical acetone after using so as to be clean for the next time of use. All the solutions were made up fresh before dosing.

The density of malathion was found to be 1.20 grams per ml. When making the solutions the corresponding volume to the required weight was measured out with a pipette and a rubber suction bulb. With larger volumes of malathion a 5 ml. pipette was used and with small volumes a 1 ml. pipette was used. The malathion was run directly from the pipette into the volumetric flask. Then corn oil was added to the flask with a dropper pipette until the flask was filled to the volumetric mark. The flask was then stoppered and the solution mixed by turning the flask upside down and back to normal position several times.

The EPN solutions were made up by first taring a 5 or 10 ml. volumetric flask on a analytical balance. Then the desired amount of EPN was weighed out by putting it into the flask with a stainless steel spatula. In order to get the EPN to dissolve two or three ml. of corn oil were added and the flask was

then warmed until the EPN melted (EPN melting point about 36° C.). The mixture was mixed and cooled before the remaining oil was added.

Dosing Equipment and Materials

The dosing was done with a 1/4 cc. B-D YALE Tuberculin syringe and an 18 gage blunt curved needle. The needles were made blunt with a file and then the point was rounded and made smoother with a silicon carbide stone. The needle point was then made very smooth by polishing it on glass. At the beginning a 1 3/4-inch needle was used. This needle was not long enough to always penetrate the cardiac sphincter in order to discharge the contents into the stomach. So a three-inch needle was used for most of the experiments.

Before dosing, the mice were anesthetized in an ether chamber. The ether chamber was composed of a large mouth Kerr pint jar and a paper lid from a paper ice cream carton. Stappled onto the inside of the lid was a wad of cotton onto which the ether was poured. The ether used was glass distilled diethyl ether which was kept in brown bottles containing a little hydroquinone as an antioxidant.

Preparation for Treatment

In preparation for treatment the mice were put into the screen topped gallon jars with feed and a water supply. Within a few hours before dosing, the mice were weighed to the nearest 0.1 of a gram. They were weighed in a tared 1/2 pint paper ice cream carton on a triple beam balance with a 111 gram capacity and a sensitivity of 0.01 gram.

The dosage levels were expressed in mg. of insecticide per kg. of body weight. The dose of insecticide that each mouse was to receive at its particular dosage level was calculated after weighing. The exact volume of solution

each mouse would receive was also calculated, based on the solutions to be made up, the average volume being 0.1 ml. When the amount of insecticide per mouse at all levels was similar, only one solution was made up. When there were great differences in quantity of insecticides per mouse at the different levels as many solutions as were needed were made up, so as to keep the volume of the doses as near to 0.1 ml. as possible.

After the solutions were made, the syringe was assembled and filled with solution and emptied several times to wet the entire plunger to eliminate any air bubbles when the syringe was filled.

Method of Treatment

The syringe was filled to the correct volume with the insecticide solution. The mouse which was to receive that particular dose was put into the ether bottle containing evaporated ether. As soon as the mouse became unconscious it was removed from the bottle and dosed. If left in the ether atmosphere over ten seconds after becoming unconscious it was found that occasionally they failed to regain consciousness. The dosing was accomplished by holding the mouse by the back of its neck and introducing the needle into the mouth and down the esophagus into the stomach. The dose was thus injected into the stomach with the syringe. The mouse usually regained consciousness within a few seconds at which time it was placed back into the holding jar. The holding jars with mice were returned to the mouse rearing room after all mice were dosed.

Determination of LD_{50}'s

The treated mice that died after dosing were removed and the observation recorded. Almost all of these mice died within one day after dosing. Occasionally one would die early during the second day. The mice that remained alive about three days after dosing were declared as having survived.

In order to determine the dosage levels to be used in the LD_{50} determinations, the approximate range was found in the literature. Several points were picked which would completely cover the range of zero to 100 per cent mortality. Two mice were dosed at each of these points. After these results were obtained several points were set up between the point where 0 per cent were killed and the point where 100 per cent mice were killed. If the mortality of the mice dosed at these points ranged from a low per cent to a high per cent these points were used with more animals for the dosage levels to determine the LD_{50}'s. If the mortality range was not covered completely, points were added as needed. All the mice for each level were pooled. The total mice dosed per point ranged from 8 to 26. These per cents were converted into probits and were plotted vs. logarithm dosage level on logarithmic-probit paper. An eye-fitted line was drawn through these points. The dosage level corresponding to the point where the line crossed the probit of five was rounded off and considered to be the approximate LD_{50}. All the dosage levels and LD_{50}'s were expressed as mg. of insecticide per kg. of body weight. These approximate LD_{50}'s served as the basis for the remaining experiments. All the LD_{50}'s and their 95 per cent fiducial intervals were later calculated on an IBM 650 computer (Sokal, 1958).

Determination of LD_{50} of a Combination of Two Insecticides

The LD_{50} of a combination of the two insecticides (malathion and EPN) was determined as described previously. The dosages consisted of a combination of a per cent of the approximate LD_{50} of malathion and the same per cent of the approximate LD_{50} of EPN. For example a dose could be a mixture of 500 mg. malathion and 9.5 mg. EPN each of which are 25 per cent of their individual LD_{50}'s. The dosage levels were set up and the LD_{50} was determined. These were all expressed as per cents of the LD_{50}'s both per cents being the same.

As mentioned previously the two solutions were made up separately and the average volume of each of the two solutions was 0.05 ml. per mouse. These two insecticides were administered separately, one immediately following the other while the mouse was still unconscious.

Arrangement for One-Dose-Treatment Reproductive Study

In the reproductive experiments only female mice were treated in order to keep the studies reduced sufficiently for one person to handle.

Three dosage levels were used. The highest level was the approximate LD_{50} (2500 mg./kg.) of which the surviving mice were used in the experiment. The middle level was 1/2 LD_{50} (1250 mg./kg.) and the low level was 1/4 LD_{50} (625 mg./kg.).

The mice at each dosage level occupied two cages. At the beginning of the experiment each cage contained six females and one male. Of these six females, four were treated and two were controls. This gave eight treated and four controls at each dosage level.

The treated females were all dosed with the one dose of malathion plus corn oil at the beginning of the experiment. At the same time the control

females were dosed with 0.1 ml. of corn oil. Three days later the males were put in with the females. If a cage of mice did not have any litters born after a few weeks the male was replaced by a new one. At the end of the 19th week, after dosing, the males were removed. At the end of the 25th week, after dosing, no more data were collected. Later the mice were sacrificed and some of the organs were weighed.

Arrangement for Chronic-Treatment Reproductive Study

In the chronic experiments four groups were used. Each group was composed of two cages each containing four treated females, two control females, and one male. One group received two high doses of malathion a week (on Wednesdays and Saturdays) each dose of which was 526 mg./kg. (21 per cent LD_{50}). In addition this group received one dose of 5 mg./kg. (19.3 per cent LD_{50}) of EPN per week, dosed on a separate day (Mondays). The other three groups received only malathion twice a week (Wednesdays and Saturdays). The high level was 526 mg./kg. (21 per cent LD_{50}) per dose; the middle dose was 263 mg./kg. (10.5 per cent LD_{50}) per dose; and the low dose was 132 mg./kg. (5.3 per cent LD_{50}) per dose. The control females were dosed with 0.1 ml. corn oil each time mice were treated. All these doses were based on the weight of the mouse at the beginning of the experiment.

The males were put in with the females three days after the treatments started and were replaced if no litters were produced within several weeks. The dosing period was ended after 19 weeks, at which time the males were removed. The experiment was terminated at the end of 25 weeks. Later the animals were sacrificed and some of their main organs were weighed.

In both the one dose and chronic experiment the adult females were weighed once a week.

Care and Observations of Litters

When signs of pregnancy, an enlarged abdomen, were noticed the pregnant mice were removed from the cages and placed into a holding jar (Fig. 2), one mouse per jar. The size and weight of the litters were recorded at least weekly between birth and weaning. The young were weaned at three weeks of age. After weaning the young were disposed of and the adult female was put back into the cage with the male.

RESULTS AND DISCUSSION

LD_{50} Determinations

Results of the LD_{50} determination are given in Table 1. The approximate LD_{50}'s fell well within the 95 per cent fiducial intervals. It was observed that the LD_{50}'s of the female mice were higher than the LD_{50}'s for male mice in the case of both compounds. The LD_{50}'s in relation to malathion for the different sexes were significantly different at the .05 level. The EPN LD_{50} for female mice show a greater range than in the males although the LD_{50} differences between sexes were significant. The differences in the lethal action of EPN on male and female mice were not as great as those observed with malathion. The LD_{50}'s were determined experimentally instead of using values found in the literature since different techniques were employed and different insecticide samples were used.

LD_{50} of Insecticide Combination

The LD_{50} of a combination of malathion and EPN was about 13 per cent of the LD_{50} of each compound. This was 260 mg. of 95 per cent malathion per kg.

and 5.0 mg. EPN per kg. These results are not much different from the ones determined by Frawley, et al. (1957) on rats.

Table 1. LD_{50}'s of white mice dosed orally with Malathion and EPN, with corn oil as a carrier.

Sex of mouse	Insecticide	Approximate LD_{50}, mg./kg., used in experiments	IBM calculated LD_{50}, mg./kg.	IBM calculated, 95% fiducial intervals, mg./kg.
Male	95% Malathion	2000	1946.0	1674.7 - 2207.3
Female	95% Malathion	2500	2457.4	2223.0 - 2945.0
Male	Crystalline EPN	38.5	38.01	35.95 - 40.55
Female	Crystalline EPN	42.0	41.90	36.79 - 45.77

The dosage levels were very close together and the mortality increase per increased unit of dosage was not consistant. There were seven dosages used between 11.75 per cent and 13.50 per cent of the LD_{50}'s of the two compounds. During the preliminary range finding there was zero mortality at dosages at the combination of 11.1 per cent of the individual LD_{50}'s. There was 100 per cent mortality in dosages of the combination of 14.3 per cent of the individual LD_{50}'s. These data indicate a high value for the slope of the regression line.

One Dose Treatment

The average number of young born and weaned per litter is given in Table 2.

A comparison was made between the size of litter of treated mice and their controls. Probabilities of differences were calculated by a "t" test adapted for unequal sample size given in Snedecor (1956). The probability of a difference in litter size between treated and control are given in Table 3. Most of these probabilities are too low to indicate a real difference. However, the size of litters at birth in both the middle and low dose have probabilities that could be significant. Their probabilities suggest that 70 to 80 per cent of the time there could have been a difference between treatment and control.

In the case of the middle dose the controls had larger litters and in the case of the low dose the treated had the larger litters. From this it would suggest that in the middle dosage level the treatment caused the mice to give birth to smaller litters. It appears at the low dosage level the treatment encouraged birth of larger litters. It is possible that this could be due to chance and not to treatment.

Table 2. Average size of litters from female white mice treated with one dose of malathion.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)
Average no. young born per litter	8.50	8.78	8.44	9.67	8.60	7.29
Average no. young weaned per litter	8.31	7.67	8.17	8.40	7.75	7.29

*1(a) - 8 female mice receiving high dose of 2500 mg. malathion/kg. in corn oil.
1(b) - 4 female mice, controls for 1(a), receiving control dose of corn oil.
2(a) - 8 female mice receiving middle dose of 1250 mg. malathion/kg. in corn oil.
2(b) - 4 female mice, controls for 2(a), receiving control dose of corn oil.
3(a) - 8 female mice receiving low dose of 625 mg. malathion/kg. in corn oil.
3(b) - 4 female mice, controls for 3(a), receiving control dose of corn oil.

Table 3. Probability that a difference exists between litter size of one dose malathion treated, female, white, mice and their controls.

Dose*	High	Middle	Low
Litter size at birth	$.10 < .20$	$.70 < .80$	$.70 < .80$
Litter size at weaning	$.40 < .50$	$.10 < .20$	$.30 < .40$

*For dosage details see footnote of Table 2.

The average weights of the litters were calculated and these data are given in Table 4.

Table 4. Average litter weights (in grams) from female mice receiving a single dose of malathion.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)
Average litter weight at birth	13.39	13.55	11.82	14.35	12.40	11.06
Average litter weight at weaning	74.56	66.18	65.03	68.04	61.70	60.74

*For dosage details see footnote of Table 2.

The litter weights were analyzed statistically by a one way analysis of variance (Snedecor, 1956) to test the possible difference between the litter weights of the treated and the litter weights of their controls. These probabilities are given in Table 5.

Table 5. Probability of a difference between litter weights of the one dose treated and the litter weights of their controls.

Dose*	High	Middle	Low
Litter weight at birth	none	.80 $<<$ .90	none
Litter weight at weaning	.80 $<<$ .90	none	none

*For dosage details see footnote of Table 2.

It appears that at the high dosage level the litters from the treated weighed more than the controls' at weaning. At the middle dosage level it appears that the control litters weighed more at birth than the litters from the treated. In both these cases it is possible that this difference could be due to chance and not to treatment since all the other weight differences were non-significant. In the other cases no weight differences were detected. These results suggest that a one dose treatment of malathion did not affect the litter weight of these mice.

In many litters a few or all of the young died or were eaten between the time of birth and weaning. Table 6 gives the ratios of number of litters weaned to number of litters born at each dosage level and number of young weaned to number of young born. This table shows a general comparison of the survival of the litters and the young. These ratios were not analyzed statistically although the size of litter and number of young were analyzed.

Table 6. Ratio of young weaned to young born from female white mice receiving one dose of malathion.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)
Ratio no. litters weaned to no. litters born	.929	1.000	.667	.833	.800	1.000
Ratio no. young weaned to no. young born	.908	.885	.645	.724	.721	1.000

*For dosage details see footnote on Table 2.

The average number of litters born and weaned per female mouse and the average number of young born and weaned per female mouse were calculated. These data are given in Table 7. It is noticed in this table that the values for the controls, in every case, were larger than the values for the treated.

A comparison was made between the number of young born and weaned per treated female and the number of young born and weaned per control female. The probability of a difference between treated females' young born and weaned and control females' young born and weaned was determined by a "t" test for unequal sample size. These probabilities are given in Table 8.

In all groups the controls had more young born and more young weaned than did the treated. In the high dosage the probabilities stayed the same at a low level of probability. In the middle dosage the probability is greater than at

the other two levels. In the low dosage there was a very low probability of a difference at birth, but by weaning time the probability of a difference had increased to between 0.60 and 0.70.

Table 7. Average reproduction per female white mouse treated with one dose of malathion.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)
Average no. litters born per mouse	1.75	2.25	1.13	1.50	1.25	1.75
Average no. litters weaned per mouse	1.63	2.25	0.75	1.25	1.00	1.75
Average no. young born per mouse	14.88	19.50	9.50	14.50	10.75	12.75
Average no. young weaned per mouse	13.50	17.25	6.13	10.50	7.75	12.75

*For dosage details see footnote of Table 2.

Table 8. Probability that a difference exists between the number of young per treated, female, white, mouse receiving one dose of malathion and their controls.

Dose*	High	Middle	Low
No. young born	$.50 \ll .60$	$.70 \ll .80$	$.20 \ll .30$
No. young weaned	$.50 \ll .60$	$.70 \ll .80$	$.60 \ll .70$

*For dosage details see footnote of Table 2.

From these probabilities it appears that at the high dosage level the treatment could have caused the treated to have less young at birth and at weaning. The probabilities for the middle dosage level strongly suggests that the

treatment was the cause for the smaller number of young. At the low level the number of young born per mouse appears to be the same, but at weaning it suggests that the treatment may be responsible for the decrease.

A comparison was made between treated and control by using the average values in Table 7. The value for the treated was divided into the value for the control. These ratios are given in Table 9.

Table 9. Reproductive comparison per mouse expressed as the ratio of the control to the one dose treatment.

Dose*	High	Middle	Low
Litters born	1.29	1.33	1.40
Litters weaned	1.38	1.67	1.75
Young born	1.31	1.53	1.19
Young weaned	1.28	1.71	1.65

*For dosage details see footnote of Table 2.

A value of one indicates that the reproduction of the treated and controls would be the same. A value of less than one indicates the treated are more productive while a value larger than one indicates the controls are more productive.

Some of the values are considerably larger than one while others are only slightly larger than one, nevertheless all the values exceed one. This consistently higher than one ratio strongly suggests that the one dose malathion treatment reduced the reproductivity of these mice.

Chronic Treatment

The average number of young born and weaned per litter of the chronically treated mice is given in Table 10.

A comparison was made between the litter sizes of the treated with their

controls using a "t" test for unequal sample size. The probability that there was a difference in litter size between the treated and their controls is given in Table 11.

Table 10. Average size of litters from chronically treated, female, white, mice.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)	4(a)	4(b)
Average no. young born per litter	5.78	8.75	8.75	8.50	7.78	7.60	7.71	8.11
Average no. young weaned per litter	6.00	8.25	7.15	6.14	7.14	6.20	7.20	7.29

*1(a) - 8 female mice receiving high dose of malathion (526 mg./kg./dose) twice a week plus 5 mg./kg. EPN once a week on different day.
1(b) - 4 female mice, controls for 1(a), receiving 3 control doses of corn oil per week.
2(a) - 8 female mice receiving high dose of malathion (526 mg./kg./dose) twice a week.
2(b) - 4 female mice, controls for 2(a), receiving 2 control doses of corn oil per week.
3(a) - 8 female mice receiving middle dose of malathion (263 mg./kg./dose) twice a week.
3(b) - 4 female mice, controls for 3(a), receiving 2 control doses of corn oil per week.
4(a) - 8 female mice receiving low dose of malathion (132 mg./kg./dose) twice a week.
4(b) - 4 female mice, controls for 4(a), receiving 2 control doses of corn oil per week.

Table 11. Probability that a difference exists between the litter size of the chronically treated, female, white, mice and their controls.

Dose*	High + EPN	High	Middle	Low
Litter size at birth	$.70 << .80$	$.10 << .20$	$.10 << .20$	$.30 << .40$
Litter size at weaning	$.70 << .80$	$.70 << .80$	$.50 << .60$	$.20 << .30$

*For dosage details see footnote of Table 10.

In the low malathion dosage level the controls had slightly larger litters both at birth and weaning, than the treated, but the differences were too slight to be detected. This suggests that the chronically administered low malathion doses had no effect on the litter size of mice both at birth and weaning.

In the high and middle levels of malathion the litter size of the control and treated was very much the same at birth. This would imply that the treatment had no effect on litter size at birth. At weaning there appears to be a possible difference, especially in the high dosage. At these two dosage levels, high and middle, the larger litters came from the treated females. From this it appears that the treatment caused better than normal survival of the litter to weaning time. It is possible that this could be due to chance and not to the treatment.

The probabilities that controls gave birth to and weaned larger litters than the mice treated with a high level of malathion and EPN is close to significance. In addition to the EPN these mice received the same amount of malathion as those in the high malathion dosage level. The chronic administration of the EPN and malathion combination appears to have an adverse effect.

The average litter weights of the chronically treated mice were calculated and are given in Table 12.

Table 12. Average litter weights (in grams) of chronically treated, female, white, mice.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)	4(a)	4(b)
Average litter weight at birth	10.07	12.93	12.97	11.40	12.24	11.50	10.80	12.28
Average litter weight at weaning	57.47	70.92	59.32	48.98	70.64	57.70	67.58	62.81

*For dosage details see footnote of Table 10.

The litter weights were analyzed statistically by a one way analysis of variance (Snedecor, 1956) to test the possible difference between litter weights of the treated and litter weights of their controls. These probabilities are given in Table 13.

Table 13. Probability of a difference between litter weights of the chronically treated and litter weights of their controls.

Dose*	High + EPN	High	Middle	Low
Litter weight at birth	none	$.75 < .80$	none	none
Litter weight at weaning	$< .75$	$.80 < .90$	$.80 < .90$	none

*For dosage details see footnote of Table 10.

At the high malathion plus EPN level it appears that the controls had heavier litters at weaning suggesting that possibly this chronic treatment was responsible for a slower gain in the weight of the young. The definite probability interval was not obtained due to unavailability of low "F" tables.

The litters of the treated, at birth and weaning, of the high malathion level were heavier than the controls indicating that this chronic treatment caused birth of heavier litter and greater weight gains to weaning.

At the middle dosage level the treated had heavier litters at weaning than their controls. This possibly means that this chronic treatment caused greater weight gains to weaning. In the other cases no weight differences could be detected.

In many litters, as in the case of the one dose treatment, a few or all of the young dies or were eaten between the time of birth and weaning. Table 14 gives the ratios of number of litters and young weaned to number of litters and young born at each dosage level. This table shows a general comparison of the survival of the litter and young.

Table 14. Ratio of young weaned to young born of chronically treated female white mice.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)	4(a)	4(b)
Ratio no. litters weaned to no. litters born	.667	1.000	.813	.875	.778	1.000	.714	.778
Ratio no. young weaned to no. young born	.692	.943	.664	.632	.714	.816	.667	.699

*For dosage details see footnote of Table 10.

In the group receiving high doses of malathion and EPN (1(a)) the ratios for the controls (1(b)) were much higher than the treated. In most of the other levels there was not much difference between the controls and treated. In all the ratios except the ratio of the number of the young of the group receiving high doses of malathion (2(a) and 2(b)) the controls had the higher ratios. These ratios were not analyzed statistically although the size of litter and number of young were analyzed.

The average number of litters born and weaned per female mouse and the average number of young born and weaned per female mouse were calculated. These data are given in Table 15.

From this table it can be seen that the number of litters born per mouse within each group was almost the same except in the low dosage level (4(a) and 4(b)) in which the controls had considerably more. In all cases the controls weaned more litters than the treated. The numbers of young born and weaned per mouse were greater in the controls than in the treated in all the dosage groups except the high malathion dosage level (2(a) and 2(b)).

Comparisons were made between the number of young born and weaned per treated female and the number of young born and weaned per control female. A "t"

test was used which was adapted for unequal sample sizes. The probabilities that there were differences in number of young born and weaned per mouse between treated females and their controls are given in Table 16.

Table 15. Average reproduction per chronically treated female mouse.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)	4(a)	4(b)
Average no. litters born per mouse	1.13	1.00	2.00	2.00	1.13	1.25	1.75	2.25
Average no. litters weaned per mouse	0.75	1.00	1.63	1.75	0.88	1.25	1.25	1.75
Average no. young born per mouse	6.50	8.75	17.50	17.00	8.75	9.50	13.50	18.25
Average no. young weaned per mouse	4.50	8.25	11.63	10.75	6.25	7.75	9.00	12.75

*For dosage details see footnote of Table 10.

Table 16. Probability that a difference exists between the number of young per chronically, treated, female, white mouse and their controls.

Dose*	High + EPN	High	Middle	Low
No. young born	.30 << .40	.10 << .20	.10 << .20	.50 << .60
No. young weaned	.60 << .70	.10 << .20	.20 << .30	.50 << .60

*For dosage details see footnotes of Table 10.

In all the groups except the high malathion the controls had the larger number of young. In the high malathion level the difference was not significant indicating that this treatment had no effect on the number of young born or weaned per mouse.

In the high malathion plus EPN group the number of young weaned approached significance. This suggests that even though there is not much difference in number of young at birth the treatment reduces the number of young per mouse by weaning time. This could possibly be due to the addition of EPN.

The probability of a difference in the middle group was too slight to be significant indicating that this level of chronic treatment had no effect on the number of young per mouse.

The probability of a difference in the low group suggests that significance might be possible. This suggests that the chronic low malathion dosage possibly causes reduction in number of young per mouse at birth and weaning.

Comparisons were made, as in the one dose treatment, between treated and control by using the average values in Table 15. The value for the treated was divided into the value for the control. These ratios are given in Table 17.

Table 17. Reproductive comparison per mouse expressed as the ratio of the control to the chronic treatment.

Dose*	High + EPN	High	Middle	Low
Litters born	0.88	1.00	1.11	1.29
Litters weaned	1.33	1.07	1.42	1.40
Young born	1.35	0.97	1.09	1.35
Young weaned	1.83	0.92	1.24	1.42

*For dosage details see footnote of Table 10.

As mentioned in explanation of Table 9 a value of one indicates that the reproduction of the treated and controls would be the same. A value of less than one indicates the treated are more productive while a value greater than one indicates the controls are more productive.

At the high malathion plus EPN level all ratios except the litters born are greater than one implying that the controls were more productive.

The high malathion group ratios ranged from slightly less than one to slightly more than one. This closeness to one suggests that the reproduction of the controls and the treated were the same at this chronic dosage level.

All the ratios of the middle and low dosage level are greater than one. Even though the probabilities of a difference between control and treated is small (Table 16) the consistently greater than one ratios suggest that chronically administered malathion at these two levels caused a reduction in the reproduction of these mice.

Treatment Effect on Adult Mice

During the course of the (175 day) experiment some of the female mice died. The average days survival per female mouse during this time are given in Tables 18 and 19.

Table 18. Average days survival per one dose treated, adult, female, mouse during the 25 week experiment.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)
Average days survival per adult female	166.9	175.0	169.6	158.8	153.4	175.0

*For dosage details see footnote of Table 2.

Table 19. Average days survival per chronically treated, adult, female, mouse during the 25 week experiment.

Dose*	1(a)	1(b)	2(a)	2(b)	3(a)	3(b)	4(a)	4(b)
Average days survival per adult female	146.9	152.8	161.4	159.3	139.1	101.5	120.5	157.8

*For dosage details see footnote of Table 10.

Comparing the results of Tables 7 and 8 with Table 18 it is noticed that

at the high dosage level the controls were more productive and they also lived longer. It is possible that part of the greater productivity of the controls is due to the longer survival period of the controls.

At the middle level the difference in number of young between control and treated was near significant. The average survival time of the controls were less than the treated. Possibly the difference would have been significant if the controls had lived as long as the treated.

At the low level the probability of a difference at weaning was between 0.6 and 0.7. If the controls of this group had not lived longer it is possible that this probability would have been lower.

Comparison of Tables 15 and 16 with Table 19 revealed that possibly some of the increased productivity of the controls at the high chronic malathion plus EPN level was due to their longer survival period.

At the high chronic malathion level the treated lived on the average about two days longer. This is too small a difference to affect the number of young produced per female. At this chronic dosage level the treatment apparently had no effect on the reproduction of these female mice.

The treated mice at the chronic middle level lived on the average about 38 days longer per mouse than the controls. The probability that the greater number of young per female possibly could have been increased to significance if the controls had lived as long as the treated.

The possible significance of the greater number of young of the controls at the low chronic level might have been reduced if the treated had lived as long as their controls.

At the end of the experiment the remaining adult females were sacrificed and some of their major organs were weighed. These average organ weights are given in Table 20.

Table 20. Average weights (in grams) of some of the major organs from one dose and chronically treated, female, white, mice.

Organ	Heart	Liver	Kidneys	Spleen
One Dose Treated*				
High	.183	2.018	.633	.327
Middle	.165	1.822	.527	.243
Low	.158	1.368	.512	.235
One Dose Controls	.179	2.364	.575	.389
Chronic Treatment**				
High + EPN	.173	2.108	.558	.362
High	.168	1.958	.548	.310
Middle	.195	2.248	.600	.408
Low	.185	3.193	.643	.513
Chronic Controls	.181	2.106	.616	.343

*For dosage details see footnote of Table 2.
**For dosage details see footnote of Table 10.

The controls of the treatments were treated very much the same so all the control weights for a study were averaged together.

Since errors in organ weight were very possible, only great difference in weights were looked for. There was much variation in the individual organ weights especially of the livers and the spleens. The average organ weights of the treated, in most cases, were not very much different from the controls. At the low one dose treatment some of the organ weights are considerably lower than the controls. This is possibly due to some of the smaller than average mice at this treatment level. At the low chronic dosage level the treated had a considerably higher average liver and spleen weight. This increase was possibly caused by one mouse which had an enlarged spleen and enlarged liver containing a large tumor (2.5 cm. across).

CONCLUSIONS AND SUMMARY

The purpose of these experiments was to find the acute toxicity of two insecticides separately and in combination, and to find what effect an insecticide might have on the reproduction of a mammal. The mammal used was the white mouse. The insecticides used were malathion and EPN, both organic phosphorus compounds.

The LD_{50}'s of malathion and EPN were determined by oral dosing of corn oil solutions of the insecticide. The approximate LD_{50}'s determined with 95 per cent malathion were 2000 mg. per kg. in males and 2500 mg. per kg. in females. The approximate LD_{50}'s of crystalline EPN were 38 mg. per kg. in males and 42 mg. per kg. in females. In both cases the LD_{50}'s of the females were significantly higher than in the males.

The LD_{50} of a combination of insecticides (malathion and EPN) of which more than additive effect was suspected was determined in male mice. The LD_{50} was determined to be about 13 per cent of each of the LD_{50}'s which amounted to 260 mg. of 95 per cent malathion and 5.0 mg. of crystalline EPN per kilogram of body weight. If one of these compounds is expressed in terms of the other their sum would amount to a sublethal dose indicating some potentiation between these two compounds.

Two separate reproduction studies were made on mice receiving insecticide dosages. In one study the female mice received one acute dose of 95 per cent malathion. In the other study the insecticides were administered chronically. In both studies comparisons were made between the treated and their controls. Comparisons were made of the litter size, litter weights, survival of young, and litters and young per mouse.

On the one dose experiment three dosage levels of malathion were used. The high dose was 2500 mg. per kg. (LD_{50}); the middle dose was 1250 mg. per kg. (1/2 LD_{50}); the low dose was 625 mg. per kg. (1/4 LD_{50}).

An analysis of the litter sizes indicated that the probability of a difference because of treatment was not very high (Table 3). It was concluded that the one dose treatment did not greatly affect the litter size at birth or at weaning.

The litter weights were higher in treated in one case and higher in controls in another case (Table 5). In the remainder of the cases no differences were detected. It was concluded that these one dose treatments did not affect the litter weight at birth or at weaning.

The survival ratios of the young given in Table 6 are higher in the controls in all cases except one. They were not analyzed statistically, but this consistantly higher ratio in the controls suggests that a smaller per cent of the litters and young born to the treated will be alive by weaning. This implies that these one dose malathion treatments might cause reduction in survival of the young between birth and weaning.

When the productivity per mouse was calculated it was observed that in every case the controls gave birth to and weaned more litters and gave birth to and weaned more young per mouse than the treated (Tables 7 and 9). The statistical analysis of the number of young born and weaned per mouse indicated that in the high and middle dosages the difference in the number born per mouse was nearing significance (Table 8). At weaning all three levels suggested that the difference might be significant. This indicates that these treatments caused reduction in reproduction per mouse.

It was concluded that these acute one dose malathion treatments did not affect the litter size and weight. However, it was concluded that these treatments were possibly responsible for the reduction in young survival between birth and weaning and the reduction in the number of litters born and weaned per mouse and number of young born and weaned per mouse. No definite decrease in difference between control and treated was noticed in the lower dosages. It was noticed that in the high dose there was less difference between control and treated. This could possibly be due to superior mice being selected since these treated mice were the survivors of an LD_{50} dose.

On the chronic experiment four dosage levels were used, all receiving malathion twice a week. At one level the mice received 5 mg. EPN per kg. once a week in addition to high malathion doses (526 mg. per kg. per dose). Another level was high malathion alone (526 mg. per kg. per dose). The middle dosage level was 263 mg. malathion per kg. per dose and the low dosage level was 132 mg. malathion per kg. per dose.

Analysis of litter size (Tables 10 and 11) showed that at the high malathion plus EPN level the controls had larger litters at birth and weaning. The difference was near significance. At the high and middle levels the treated weaned larger litters of near significance. In the other cases no difference was detected. It could possibly be concluded that the malathion chronically administered caused the increase in litter size. When EPN was added it appeared that the treatment reduced litter size.

The analysis of litter weights (Tables 12 and 13) gave very much the same results as in the litter sizes.

In a comparison of the survival ratios (Table 14) it was noticed that in all but one case the litters and young from the controls had slightly higher

survival. It does imply that these chronic treatments might have a slight effect on decreasing the survival of the litters and young.

By statistical analysis it was noticed that it was possible that more young per control mouse were weaned at the high malathion plus EPN level (Tables 15, 16, and 17). Also it was suggestive that at the low level more young were born and weaned by controls.

Comparing production per mouse and survival of the adults (Table 19), it is possible that the controls in the middle level could have been more productive if they had lived as long as the treated. At the low level the reproduction of controls and treated could have been more the same if the adult survival would have been the same.

The treated females receiving the chronic high malathion level showed in some cases greater productivity than their controls. Since the controls and treated were picked at random, it is possible that the stronger and more prolific mice were put into the treated group.

When the survival of the adults was taken into account, it was concluded that these chronic treatments of malathion did not decrease the litter size and weight, or the survival of the young, or the productivity per female mouse. It was concluded that the addition of EPN to the diet possibly caused reduction in size and weight of litter, survival of young, and productivity per female mouse.

The one dose and chronic treatments did not appear to have any great effect on the organ sizes of the adult mice.

This work was in no way conclusive. Much more work of this nature could be done before arriving at definite and conclusive results. It is believed that this work will stimulate continued investigations of this nature and open new phases of toxicological research.

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SOME EFFECTS OF MALATHION AND EPN ON MICE

by

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The use of organic phosphorus insecticides for insect control is becoming very common. Since mammals are also often exposed to insecticides, mammalian toxicity studies must be made before a compound can be put on the market. Even than, experimental work must continue. In this thesis, the LD_{50}'s of malathion and EPN were determined, and in addition an LD_{50} of a mixture of malathion and EPN was determined, since it is possible that an animal can come into contact with several insecticides. Reproduction studies were conducted with malathion, both acute and chronic, and with a combination of malathion and EPN to see if the fertility of the treated was affected and to see if the survival and size and number of the young was altered.

The experimental animals were Swiss white mice. The mice were provided with an ample supply of Purina Laboratory Chow and water.

The insecticides used were 95 per cent malathion, 0,0-dimethyl S-(1,2-dicarboethoxyethyl) dithiophosphate, and crystalline EPN, 0-ethyl-0-p-nitrophenyl benzenethiophosphate.

The insecticide solutions, using corn oil as a carrier, were made up to a concentration so that the average dose volume would be about 0.1 ml. The dosing was accomplished by anesthetizing with diethyl ether and then dosing orally with an 18 gage curved blunt needle and 1/4 cc. syringe.

LD_{50}'s were determined by finding the range from zero to 100 per cent mortality and then dosing groups of mice at several different levels within this range. The results were plotted on log-probit paper and also calculated on the IBM 650. The approximate LD_{50} for 95 per cent malathion in male mice was 2000 mg. per kg.; for females it was 2500 mg. per kg. For crystalline EPN the approximate LD_{50} in male mice was 38 mg. per kg.; for females it was 42 mg. per kg. The LD_{50}'s of the females were significantly larger, at the 5 per cent level, than the males.

The LD_{50} of a combination of malathion and EPN was about 13 per cent of the LD_{50} of each compound. This amounted to 260 mg. of 95 per cent malathion per kg. and 5.0 mg. of crystalline EPN per kg. The sum of these two in terms of the other is a sublethal dose. This indicates that there possibly is potentiation between these two compounds.

In the acute one-dose reproduction study three dosage levels of malathion were used. The highest level was 2500 mg./kg. (LD_{50}); the middle level was 1250 mg./kg. (1/2 LD_{50}); the low level was 625 mg./kg. (1/4 LD_{50}). At each level, eight females were treated and four females and two males served as untreated controls. Comparisons were made of the litter size, litter weights, survival of young, and litters and young per mouse. Part of the results was analyzed statistically.

It was concluded that the acute one dose malathion treatments did not affect the litter size and weight, but they were possibly responsible for reduction in young survival and reduction in the number of litters born and weaned per mouse and the number of young born and weaned per mouse. It was noticed that with the high dose, there was less difference between control and treated. This could possibly be due to selection of superior mice in the treated since these were the survivors of an LD_{50} dose.

In the chronic experiment, four dosage levels were used. All received malathion twice a week (on Wednesdays and Saturdays). At one level the mice received 5 mg. EPN per kg. once a week, on a separate day (on Mondays), in addition to high malathion doses (526 mg. per kg. per dose). Another dosage level consisted of high malathion alone (526 mg. per kg. per dose). The middle dosage level was 263 mg. malathion per kg. per dose and the low dosage was 132 mg. malathion per kg. per dose. In this experiment, as in the one dose

experiment, comparisons were made of the litter size, litter weights, survival of young, and litters and young per mouse. Part of these results were analyzed statistically.

The results of this study were variable. When the survival of the adults were taken into account it was concluded that the chronic treatments of malathion did not decrease the litter size and weight, or the survival of the young, or the productivity per female mouse. It was concluded that the addition of EPN to the chronic malathion treatment possibly caused reduction in size and weight of litter, survival of young and productivity per female mouse.

The one dose and chronic treatments did not appear to have any great effect on the organ sizes of the adult mice.

In both the one dose and chronic treatments there were not enough animals used to statistically detect a slight difference between treated and control. In many instances the average results were suggestive that the treatment reduced productivity and was harmful to the young, but the differences were statistically non-significant. More work of this nature should be done before definite conclusions could be reached.